

OFFICE OF NAVAL RESEARCH  
CONTRACT N00014-88-C-0118

TECHNICAL REPORT 94-09

WHOLE BLOOD VISCOSITY IN INDIVIDUALS WITH INSULIN-DEPENDENT  
DIABETES MELLITUS:  
EXPLORING THE ROLE OF THE POROUS BED VISCOMETER

BY

M.S. ROSENBLATT, C.R. VALERI, E.W. MERRILL, J.P. CROWLEY, AND  
F.W. LOGERFO

NAVAL BLOOD RESEARCH LABORATORY  
BOSTON UNIVERSITY SCHOOL OF MEDICINE  
615 ALBANY STREET  
BOSTON, MA 02118

30 JUNE 1994

Reproduction in whole or in part is permitted for any purpose of  
the United States Government.

Distribution of this report is unlimited.

DTIC QUALITY INSPECTED 2

19990225171

## ABSTRACT

**Title:**

Whole blood viscosity in individuals with insulin-dependent diabetes mellitus: Exploring the role of the porous bed viscometer

**Objective:**

The study was performed to evaluate the capacity of a porous bed viscometer (PBV) to demonstrate differences in blood viscosity in patients with diabetes mellitus and to investigate the role that various cellular and plasma components play in determining blood viscosity at physiologic shear rates.

**Research, Design and Methods:**

A prospective, technician-blinded study comparing recruited insulin-dependent diabetic patients and non-diabetic control individuals was performed at a tertiary-care referral hospital and its associated outpatient clinic. Thirty insulin-dependent diabetic individuals were compared with twenty-three non-diabetic individuals. Blood specimens were drawn from all individuals in a similar fashion and samples were analyzed in a blinded fashion for a panel of serum and cellular components and blood viscosity utilizing a PBV.

**Results:**

The PBV was able to demonstrate that differences in hematocrit are responsible for the majority of blood viscosity variability. Diabetic status played a small but statistically significant role in explaining some of the variability in blood viscosity, as did fibrinogen.

**Conclusions:**

This study supports previous reports which suggest that individuals with insulin-dependent diabetic mellitus have a higher blood viscosity than should be expected on the basis of hematocrit and fibrinogen alone. In addition, the study demonstrates that the PBV is a sensitive device, providing reproducible measurements of whole blood viscosity at low shear rates. The porous bed viscometer could be used for large numbers of examinations with relative ease of use, safety and cost.

# WHOLE BLOOD VISCOSITY IN INDIVIDUALS WITH INSULIN-DEPENDENT DIABETES MELLITUS: EXPLORING THE ROLE OF THE POROUS BED VISCOMETER

## INTRODUCTION

Abnormalities in blood viscosity have been implicated in the clinical manifestations of a number of diseases. The relationship between blood viscosity and pathophysiology is readily apparent when it represents the effect of a single blood component, such as red blood cells in polycythemia vera or fibrinogen in hyperfibrinogenemia (1). In contrast, the relationship between multiple factors such as hematocrit, plasma fibrinogen levels and blood viscosity have not been consistently demonstrated in individuals with diabetes (2,3,4,5,6,7,8).

In addition to precision, sensitivity and sample size, the methodology for measuring blood viscosity has also contributed to difficulty in analysis. The traditional technique relies on measurement of the resistance that blood exhibits when placed in a container between a rotating and stationary surface. Artifacts associated with this technique can mislead investigation (9, 10). First, separation of the cellular and plasma components of blood can occur within the container leading the viscometer to measure the viscosity of a fluid which is not uniformly mixed. Second, viscosity measurements are dependent on the shear rate applied to the fluid in question. The physiologically important shear rates are probably those which are equivalent to shear rates which are present in the microvascular tree, on the order of  $10\text{-}30\text{ seconds}^{-1}$ . Rotational devices generally are less accurate at these low shear rates.

A recently introduced device may successfully address several of these problems(11, 12, 13, 14, 15). The whole blood porous bed viscometer (PBV) is an inexpensive, disposable, simple-to-use device which has been shown to accurately estimate the viscosity of whole blood at rates

comparable results. First, the device has been demonstrated to provide accurate, reproducible measurements which discriminate between small differences in viscosity between specimens. Second, the PBV is a simplified method of measurement compared to more traditional methods and coupled with its relatively low cost-per-test allows for the study of large numbers of samples. In this way smaller differences can be statistically demonstrated by testing larger numbers of individuals. An added advantage of the PBV over more traditional devices such as the cone-plate viscometer or the capillary tube method is that the PBV measures whole blood using a shear rate around  $20 \text{ seconds}^{-1}$ , a more physiological rate, and in a manner that eliminates the problems of separation that plague rotational devices. Lastly, the disposable, self-contained design reduces risks of blood-borne infection and drastically reduces equipment maintenance.

This study was undertaken to examine the capacity of the PBV to demonstrate differences in blood viscosity in patients with patients with diabetes mellitus and to investigate the role that various cellular and plasma components play in determining blood viscosity at this shear rate.

## **MATERIALS AND METHODS**

### **Patient selection**

30 insulin-dependent diabetic individuals and 23 non-diabetic volunteers were entered into the study from both the in-patient and out-patient services at the New England Deaconess Hospital and the Joslin Diabetes Center, Boston, MA. Normal, non-diabetic individuals were recruited as volunteers from the hospital staff and from patient's families. Informed consent was obtained from all participants following guidelines of the New England Deaconess Hospital Investigational Review Board.

In addition to the data collected from these 53 individuals, historical control data from a previous study testing the characteristics of the PBV was utilized. The measurements of whole blood viscosity, hematocrit and fibrinogen on 982 healthy individuals were available for review from previous work by several of the authors (11). This larger pool was utilized to confirm the methodology of the results of this study and to compare our diabetic population to a larger pool of non-diabetic individual data.

### **Blood Collection, Storage and Analysis**

Fasting blood samples were collected into plastic syringes and stored at room temperature for periods of time not exceeding 4 hours prior to analysis. Specimens were analyzed in a blinded fashion without knowledge of patient characteristics. Specimens for red cell and white cell counts were collected in K<sub>3</sub>EDTA vacutainer tubes and were analyzed using a Coulter ZBI (Coulter Electronics). Hemoglobin concentration was determined on blood collected in K<sub>3</sub>EDTA using a Coulter hemoglobinometer. Hematocrit was determined using a standard microhematocrit method. Platelet counts were determined using manual phase microscopy. Fibrinogen levels were measured in specimens collected in K<sub>3</sub>EDTA, using the Coag-A-Mate x 2 method (General Diagnostics). Glycosylated hemoglobin was measured using an electrophoresis

method (Corning). Glucose, albumin, serum protein levels and sedimentation rates were measured using routine techniques.

Whole blood viscosity was determined in duplicate using a porous bed viscometer at 37 °C on specimens gathered in K<sub>3</sub>EDTA within 4 hours of collection. The porous bed viscometer has been described previously (11). Briefly, the viscometer measures blood flow through a bed of sintered polyolefin (porex) which has been shown to contain randomly communicating channels of a mean pore size of  $65 \pm 20 \mu\text{m}$ , by scanning electron micrographs. When blood is introduced into the porous bed from above, gravitational forces initiate flow at a rate comparable to that in the arteriolar and venule level. By recording the elapsed time that it takes for a fixed quantity of blood to flow through the bed a relative viscosity measurement in seconds is obtained. By calibration with Newtonian liquids (e.g. silicone oil) of known viscosity, the flow time in seconds can be converted to a value in units of centipoise(11).

### **Statistical Analysis**

Mean and standard deviation ( $M \pm SD$ ) are reported for each variable. Stepwise multivariate regression was used to evaluate the influence of individual variables and combinations of variables on the measurement of whole blood viscosity. A p-value of  $< 0.05$  was considered significant for all statistical procedures.

## RESULTS

The parameters measured in the primary population of fifty-three individuals are outlined in Table 1. The diabetic and normal populations were comparable from a demographic perspective, with no statistically significant differences in age or gender distribution among the two populations.

The diabetic population had significantly lower hemoglobin and hematocrit, total protein and albumin concentrations and significantly higher glucose and glycosylated hemoglobin concentrations and a higher sedimentation rate.

To verify that viscosity measurements were performed appropriately, the results from the non-diabetic individuals in this study were compared to the historical control data pool (11). Stepwise multivariate regression was performed on the independent variables and dependent variable common to both normal populations: site of study, hematocrit and fibrinogen levels which predicted whole blood viscosity. Variability in whole blood viscosity was determined to be independent of any differences in study site for the two groups.

This comparison demonstrated that the relationship between blood viscosity, hematocrit and fibrinogen could be described in all non-diabetic individuals by the following equation :

$$WBV = -23.85 + 1.07 \text{ HCT} + 0.0068 \text{ FBG}$$

where WBV=whole blood viscosity, HCT= hematocrit and FBG= fibrinogen.

( s= 3.279,  $R^2=61.1\%$ , F-test:  $p<.001$ , t-test for HCT and FBG:  $p <0.001$ )



With this confirmation of methodology, and the findings that the 23 non-diabetic individuals could not be distinguished from the larger group of 982 non-diabetic individuals from the previous study, the 30 diabetic patients could be compared to a much larger group of 1005 non-diabetic individuals, to strengthen the statistical analysis.

Isolation of the influence of diabetes on whole blood viscosity was performed in a similar fashion using stepwise multivariate regression. The addition of the variable of diabetic status in this analysis allowed for estimation of the interaction of diabetes on whole blood viscosity. The relationship between whole blood viscosity and the variables hematocrit, fibrinogen and diabetic status can best be represented by the equation:

$$WBV = - 23.7 + 1.05 \text{ HCT} + 4.21 \text{ DIAB} + 0.008 \text{ FBG}$$

where WBV=whole blood viscosity, HCT= hematocrit,  
 FBG= fibrinogen, DIAB= diabetes(1= disease, 0=normal)  
 (  $s = 0.0637$ ,  $R^2=61.5\%$ , regression F-test  $p<0.001$ ,  
 DM, HCT and FBG t-tests:  $p < 0.001$ , .)

Using stepwise linear regression the relative contribution of each variable predicting whole blood viscosity can be determined. Differences in hematocrit are responsible for 58% of the variability in viscosity. Diabetic status is responsible for an additional 2% of the variability. Although there is a statistically significant relationship between fibrinogen and viscosity, it only was responsible for an additional 1.5% of the variability in whole blood viscosity. The reduced influence of diabetic status may be related to the low number of diabetic individuals in the smaller group of patients. There is an additional 38% of variability in blood viscosity not predicted by hematocrit, diabetic status or fibrinogen level.

A broad range of variables were available for analysis in the group of 53 individuals. Measurement of cellular components of the blood included hemoglobin, hematocrit, mean

corpuscular volume, hemoglobin A<sub>1</sub>C, white cell and platelet concentration and sedimentation rate. Plasma components included fibrinogen, total protein, albumin and glucose. Diabetic status, age and gender were utilized as well. Stepwise multivariate regression demonstrated that whole blood viscosity was significantly dependent upon age, diabetic status, hematocrit concentration and albumin concentration, all at  $p < 0.05$  level. Fibrinogen concentration approached statistical significance ( $p = 0.10$ ). None of the other variables were useful in predicting whole blood viscosity over and above those variables with  $p$  values  $< 0.05$ .

## DISCUSSION

This study demonstrates that changes in hematocrit are responsible for the majority of the variability in whole blood viscosity, as measured by the porous bed viscometer. With the additional 4% of viscosity variability explained by diabetic status and fibrinogen concentration, nearly 40% of the remaining variability remains unexplained. Additional relationships between age and albumin concentration were demonstrated but with weaker statistical support due to small sample size. It is clear however that regardless of what ever other relationships are demonstrated, hematocrit is the major single factor that influences viscosity.

It has been recognized for a number of years that hematocrit levels play an important role in the development of cardiac ischemia and infarction ( 16, 17, 18). The influence that an elevated hematocrit has on the onset and propagation of ischemic strokes has also been previously recognized ( 19, 20, 21). Review of the role of hematocrit in complications related to diabetes is less clear. Studies demonstrate both an increase in hematocrit (3, 22) in those with complications of diabetes, as well as a lower average hematocrit in the diabetic population (2). The average hematocrit of the diabetic patients in this study was statistically lower than the normal population.

The whole blood viscosity of the diabetic patients was not statistically different than that of the non-diabetic individuals. However, when differences in the determinant variables of whole blood viscosity, hematocrit and fibrinogen were controlled for through multivariate regression, the difference in whole blood viscosity between diabetics and non-diabetics is clear. If one theorizes

that blood viscosity is a vital determinate of the adequacy of blood circulation, the relative anemia in the diabetic population may be a compensatory response to the increase in blood viscosity brought about by the diabetic state. The ability to control blood viscosity in a normal range, through a compensatory anemia, may be lost in some diabetics, leading to higher blood viscosity and resultant diabetic complications.

These results support previous findings that suggest that diabetic patients as a group have a higher viscosity than should be expected on the basis of hematocrit and fibrinogen alone, although the factor or factors which are responsible for this increased viscosity were not clearly identified in this study. The statistical method of stepwise multivariate regression will identify only those variables which contribute to predicting the dependent variable, in this case whole blood viscosity, over and above that which is predicted by other variables. Therefore, diabetic individuals have a factor or factors, over and above those already identified, i.e. hematocrit, age, albumin and possibly fibrinogen levels, which is responsible for the increased viscosity in the diabetic patient. Neither glucose levels themselves, nor glycosolated hemoglobin levels, can fully explain this increase, based upon the results of this study.

Evidence for the relative impact of fibrinogen concentration on whole blood viscosity is also presented. Variations in fibrinogen concentrations can be statistically linked with changes in blood viscosity, however, the statistical impact of fibrinogen is minimal, accounting for less than 2% of the variability in blood viscosity.

This study also supports previous findings which demonstrated that the porous bed viscometer is a sensitive device, providing reproducible measurements of whole blood viscosity at low shear rates of about  $20 \text{ sec}^{-1}$ . Future investigation into the role of earlier, more stringent control of diabetes may include evaluation of changes in blood viscosity with tighter hyperglycemia control. The porous bed viscometer could be used for large numbers of examinations with relative ease of use and safety and may allow for the inexpensive and accurate monitoring of blood viscosity compared to more traditional methods of viscosity determination.

TABLE 1  
DEMOGRAPHICS AND RESULTS

PARAMETERS	Diabetic	Non-diabetic	p=	Male	Female	p=
n=	30	23		37	16	
Age (Yrs.)	42.7 ± 14.3	43.0 ± 16.1	N.S.	41.4 ± 14.5	46.1 ± 15.6	N.S.
Male/ Female	19/11	18/5	N.S.	---	---	---
Hgb (gm/dl)	14.2 ± 1.3	15.6 ± 1.4	0.0006	15.3 ± 1.3	13.6 ± 1.5	0.0007
Hct (%)	41.6 ± 6.7	43.8 ± 3.3	0.031	43.8 ± 3.0	39.6 ± 3.5	0.0003
HgbA1C (%)	10.7 ± 2.9	6.2 ± 0.7	0.0001	8.4 ± 3.1	9.5 ± 3.3	N.S.
MCV (fl)	84.7 ± 9.5	84.2 ± 6.7	N.S.	84.4 ± 7.6	84.6 ± 10.1	N.S.
WBC (K/ml)	7.3 ± 3.5	5.9 ± 1.3	N.S.	6.5 ± 1.6	7.1 ± 4.6	N.S.
Platelets (K/ml)	222 ± 68	236 ± 62	N.S.	226 ± 63	232 ± 72	N.S.
Sed rate (mm/min.)	18.8 ± 15.1	11.3 ± 10.2	0.04	12.0 ± 12.4	23.8 ± 13.2	0.005
Whole blood viscosity (sec.)	25.3 ± 4.7	25.6 ± 4.5	N.S.	26.5 ± 4.2	22.8 ± 4.5	0.009
Albumin (gm/dl)	3.8 ± 0.5	4.4 ± 0.3	0.0001	4.1 ± 0.5	3.8 ± 0.4	0.04
Total protein (gm/dl)	6.5 ± 0.5	7.3 ± 1.2	0.012	6.9 ± 1.0	6.6 ± 0.5	N.S.
Glucose (mg/dl)	198 ± 100	78 ± 17	0.0001	133 ± 97	184 ± 91	N.S.
Fibrinogen (mg/dl)	299 ± 89	288 ± 76	N.S.	288 ± 79	309 ± 95	N.S.

All values as mean ± s.d.

## REFERENCES

1. Reploge RL, Meiselman HJ, Merrill EW. Clinical implications of blood rheology studies. *Circulation* 36:148-160, 1967
2. Skovberg F, Nielsen AV, Schlichtkrull J, Ditzel J. Blood viscosity in diabetic patients. *Lancet* 1:129-131, 1966
3. Lowe GD, Lowe JM, Drummond MM, et al. Blood viscosity in young male diabetics with and without retinopathy. *Diabetologia* 18:359-363, 1980
4. Hoare EM, Barnes AJ, Dormandy JA. Abnormal blood viscosity in diabetes mellitus and retinopathy. *Biorheology* 13: 21-25, 1976
5. Barnes AJ, Locke P, Scudder PR, et al. Is hyperviscosity a treatable component of diabetic microcirculatory disease? *Lancet* 2:789-791, 1977
6. Memeh CU. Differences between plasma viscosity and proteins of types 1 and 2 diabetic Africans in early phase of diabetes. *Hormone and Metabolic Research* 25:21-3, 1993
7. Schut NH, van Arkel EC, Hardeman MR, et al. Blood and plasma viscosity in diabetes: possible contribution to late organ complications? *Diabetes Research* 19:31-5, 1992
8. MacRury MS, Lockhart JC, Small M, et al. Do rheological variables play a role in diabetic peripheral neuropathy? *Diabetic Medicine* 8:232-6, 1991
9. Merrill EW. Rheology of blood. *Physiological Review* 49: 863-888, 1969
10. Ernst E. Blood viscosity- a comparative study on three rotational instruments. *Biorheology* 22: 471-475, 1985
11. Crowley JP, Valeri CR, Metzger J, et al. The estimation of whole blood viscosity by a porous bed method. *Am J Clin Path* 96: 729- 737, 1991
12. Crowley JP, Metzger JB, Merrill EW, Valeri CR. Whole blood viscosity in beta thalassemia minor. *Ann Clin Lab Sci* 22: 229- 235, 1992
13. Crowley JP, Chazan JA, Metzger JB, et al. Blood rheology and 2,3- diphosphoglycerate levels after erythropoietin treatment. *Ann Clin Lab Sci* 23: 24-32, 1993
14. Schwartz JA, Keagy BA, Johnson G. Determination of whole blood apparent viscosity: experience with a new hemorheologic technique. *J Surg Research* 45: 238-247, 1988
15. Johnson WC, Sentissi JM, Baldwin D, et al. Treatment of claudication with pentoxifylline: are benefits related to improvement in viscosity? *J Vasc Surg* 6: 211-216, 1987
16. Nicolaidis AN, Bowers R, Horbourne T et al.: Blood viscosity, red-cell flexibility, haematocrit, and plasma-fibrinogen in patients with angina. *Lancet* 2:943-945, 1977
17. Jan KM, Chien S, Bigger JT : Observations on blood viscosity changes after acute myocardial infarction. *Circulation* 51: 1079-1084, 1975

18. de Simone G, Devereux RB, Chien S et al.: Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults. *Circulation* 81: 107-117, 1990
19. Tohgi H, Yamanouchi H, Murakami M et al: Importance of the hematocrit as a risk factor in cerebral infarction. *Stroke* 9: 369-374, 1978
20. Ciuffetti G, Mercuri M, Parnetti L et al: Hemorheologic factors in the postacute phase of ischemic stroke. *Angiology* 16: 437-448. 1988
21. Kannel WB, Gordon T, Wolf PA, et al: Hemoglobin and the risk of cerebral infarction: The Framingham study. *Stroke* 3:409-420, 1972
22. Kannel WB, D'Agostino RB, Wilson PWF et al.:Diabetes, fibrinogen and risk of cardiovascular disease: the Framingham experience. *Am Heart J* 120:672-676, 1990